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Studies on the resin chemistry of some Western Australian plants

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Abstract

Chemical research on plant resins in Western Australia is reviewed with particular reference to components of the sub-family Ricinocarpoideae of the Euphorbiaceae. The diterpenoids are discussed in terms of their relationships with the gibberellin pathway. Separate correlations between the bicarbocyclic and tetracyclic groups are also given and in vivo experiments to define some of these relationships are described. Microbiological hydroxylation of tetracyclic systems is also summarised and an account of some experiments relating to gibberellin biosynthesis is presented. The occurrence of triterpene and flavonoid components associated with the resins is also given.

Introduction

As casual observers the late Professor White and I were particularly impressed by the frequency plant species in the Eremean and Wheatbelt areas of Western Australia exhibit resin coatings on their leaves and terminal branches. The organic chemist is aware not only of industrial applications of resins and allied substances but also of the fact that these materials have been one of the main sources of the polyterpenes. In particular the most abundant of the resins, those from pines, revealed the field of diterpenes to organic chemistry. At the outset of this work diterpenes were a small group of compounds which were largely confined to the pines and so we were interested to discover if these common coatings were a major extension of this group. As with the pine resins we were at first restricted by the problem of separating what were often complex mixtures and progress was accelerated by sampling on a wide scale and selecting the more tractable mixtures. Developments in analytical and preparative methods of separation allowed us to return later to the more complex cases which are commonly encountered, particularly in *Eremophila*. The project we began in this area is a continuing one but those aspects of it related to the sub-family Ricinocarpoideae of the Euphorbiaceae have reached the stage where they can be usefully reviewed. In view of developing interest by biologists in plant resins (Dell and McComb 1974, 1975) the opportunity is also taken to catalogue resin components from other local species. In the early stages the main problems lay in the proof of structure and stereochemistry. Advances in physical methods make these problems secondary and since they are of interest only to chemists this aspect of the work is treated briefly. On the other hand the biosynthesis of many of these substances is of general interest and in the sequel the structural patterns are identified and correlated through direct pathways although the existence of metabolic networks is most probable. A number of in vivo experiments have been carried out to define more obscure biosynthesis and these are discussed. The relationship of the *ent*-kaurene diterpenes to gibberellins is of particular importance and our contribution in this area is also summarised.

Resins from species of Ricinocarpoideae

The first material chosen came from the widespread *Beyeria drummondii* which gave ~20% of ether soluble resin of which almost a quarter was the cinnamate of a diterpenetriol with a new carbon skeleton which we called beyerane. It was clear that an extension to other members of the family was warranted and wide-ranging plant collections were undertaken to secure material. These provided many of the known species of Ricinocarpoideae along with a number of new members some of which were ultimately described at Kew (Airy Shaw 1971) through the agency of the Western Australian Herbarium. These included *Beyeria calycina* and *B. calycina* var. *minor*, *B. brevifolia* var. *truncata* and var. *brevipes*, and *Bertya cupressoidea*. Inevitably many of our early papers used undefined names and the relationship of these plants to the present taxonomy has been summarised recently (Errington *et al.* 1976). Most species of *Beyeria* and *Bertya* carried resin, exceptions being the coastal *Beyeria cyanescens* which lacked significant coating and the near-coastal *B. gardneri* which had very little. On the other hand *B. viscosa* from Rottnest Island had a substantial resin layer. No resin was observed on *Monotaxis* spp. or on *Ricinocarpus tuberculatus* or *R. velutinus* although the latter showed some toxicity which was associated with low-level anti-tumor activity. *Amperea* spp. have not been studied.

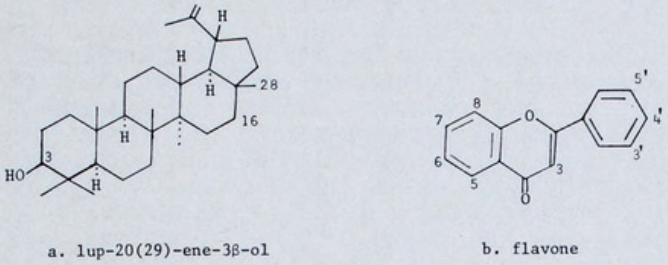
In general the resins were hard materials which consisted largely of widely varying proportions of flavonoids, diterpenes and fatty material with triterpenes appearing in *Beyeria brevifolia*, *B. viscosa* and *B. leschenaultii*. The resin of *B. brevifolia* var. *truncata* represents one extreme in that it is essentially flavonoid whereas *B. viscosa* and *B. lepidopetala* show no significant proportion of these compounds. A list of the main polyterpenoids and flavones isolated together with salient references is given in Table I.

Table 1

Chemical components of resins from species in the Ricinocarpoideae

Species	Polyterpenes (Structures)	Flavones*	References
Beyeria brevifolia (Muell. Arg.) Benth var. *brevifolia* Airy Shaw	secopimaradiene (*21*), lup-20(29)-ene-3β,16β-diol, and lup-20(29)-ene-3β,16β,28-triol	5,7,3′-trihydroxy-3,8,4′,5′-tetramethoxy-	Chow and Jefferies 1968 Errington *et al.* 1976
B. brevifolia (Muell. Arg.) Benth var. *brevipes* Airy Shaw	kauranes (*39*), (*40*)		Baddeley *et al.* 1964c
B. brevifolia (Muell. Arg.) Benth var. *truncata* Airy Shaw		5-hydroxy-7,4′-dimethoxy- 5,4′-dihydroxy-7-methoxy- 5,7-dihydroxy-3,8,4′-trimethoxy-	Dawson *et al.* 1965
B. brevifolia (Muell. Arg.) Benth var. nov.	secopimaradiene (*21*), lup-20(29)-ene-3β,16β-diol and lup-20(29)-ene-3β,16β,28-triol.	5,7,4′-trihydroxy-3,8-dimethoxy- 5,7,4′-trihydroxy-3,8,3′-trimethoxy- 5,7-dihydroxy-3,8,3′,4′,5′-pentamethoxy-	Stacey 1970
B. calycina Airy Shaw	labdanes (*10*) (*11*)(*12*/(*13*) kauranes (*8*)(*39*)(*41*)(*42*)(*45*)(*46*)	5,4′-dihydroxy-3,7-dimethoxy-	Jefferies and Payne 1965
B. calycina Airy Shaw var. *minor* Airy Shaw	beyeranes (*23*)(*25*)(*27*)(*29*) secobeyeranes (*24*)(*26*)(*30*)		Ghisalberti and Jefferies 1968, Bakker *et al.* 1972
B. drummondii Muell. Arg.	beyerol (*25*)	5,4′-dihydroxy-7-methoxy- 5,7-dihydroxy-4′-methoxy- 5,7,4′-trihydroxy-	Jefferies *et al.* 1963 Stacey 1970 O'Connell and Maslen 1966
B. drummondii Muell. Arg. var. nov.	beyeranes (*22*)(*25*)(*28*)	5,3′,4′-trihydroxy-7-methoxy- 5,3′-dihydroxy-3,7,4′-trimethoxy- 5,7-dihydroxy-3,7,8-trimethoxy-	Ratajczak 1969 Jefferies unpublished
B. latifolia (Muell. Arg.) Baill.	kauranes (*35*)(*38*)	5,4′-dihydroxy-7-methoxy- 5,7,4′-trihydroxy-	Jefferies and Retallack 1968a
B. lepidopetala F. Muell.	labdanes (*15*)(*16*)(*17*)		Coates 1966
B. leschenaultii (D.C.) Baill.	kauranes (*31*)(*35*)(*38*)(*43*)(*46*)(*48*) lup-20(29)-ene-3β,16β-diol	5,4′-dihydroxy-3,7,8-trimethoxy-	Jefferies and Retallack 1968b Baddeley *et al.* 1964a,b
B. viscosa (Labill.) Miq.	kauranes (*31*)(*32*)(*33*)(*34*)(*35*)(*36*)(*37*)(*42*) lup-20(29)-ene-3β,16β-diol lup-20(29)-en-3-one		Coates 1966
Bertya cupressoidea Airy Shaw	bertyadionol group (*50*)(*51*)		Ghisalberti *et al.* 1974 Maslen *et al.* 1975
B. dimerostigma F. Muell.		5,3′,5′-trihydroxy-3,7,4′-trimethoxy-	Henrick and Jefferies 1964b
Ricinocarpus muricatus Muell. Arg.	labdanes (*14*)(*15*)(*16*)(*17*)(*18*)(*19*)	5,7,3′,4′-tetrahydroxy-3,8-dimethoxy- 5,3′,4′-trihydroxy-3,7,8,-trimethoxy- 5,3′-dihydroxy-3,7,8,4′-tetramethoxy-	Henrick and Jefferies 1965a. 1965c
R. psilocladus (Meull. Arg.) Benth.		5,7,3′,4′-tetrahydroxy-	Stacey 1970
R. stylosus Diels	polyalthic acid (*20*); kauranes (*8*)(*41*)(*43*)(*44*)(*45*)(*47*)	5,4′-dihydroxy-3,7,8-trimethoxy- 5,3′-dihydroxy-3,7,8,4′-tetramethoxy- 5-hydroxy-3,7,8,3′,4′-pentamethoxy-	Henrick and Jefferies 1964a 1964b, 1965b

* For numbering see Figure 8b.



a. lup-20(29)-ene-3β-ol b. flavone

Figure 1.—A.- lup-20(29)-ene-3β-ol. B.- flavone.

Beyeria brevifolia, B. viscosa and *B. leschenaultii* provided large quantities of the triterpene lup-20(29)ene-3β, 16β-diol, which in the latter plant was associated with a small proportion of the 3β,16β,28-triol. Despite the fact that lup-20(29)-ene-3β-ol (Fig. 1a) and the 3β,28-diol are very widely distributed in plants neither the 3β,16-diol or the triol had been isolated previously. Evidently 16β-hydroxylation of the 3β-ol is an abberration of the hydroxylating system deflecting attack from C-28 to the neighbouring C-16. The structures of these triterpenes were proved by interrelation with each other and with known lupane derivatives.

Despite the fact that the flavones (Fig 1b) are widely distributed and well documented most of the substances we isolated were new members of the class. The resin flavonoids are exceptional in that not only are they highly methoxylated but they frequently occur as a large proportion of the plant material. A number of common structural features were observed and these include the ubiquitous 5-hydroxyl group and the fact that when substitution occurs at C-3 or C-8 then the substituent is always methoxyl. Oxygenation of the A ring follows the normal 5,7 pattern, established by assembly of malonyl CoA residues and later substitution is limited to C-8; the common alternative C-6 is not substituted. Substitution of the phenyl ring follows the normal patterns 4'; 3', 4'; 3', 4', 5'. In non-resin flavones methylation of B ring hydroxyl groups occurs almost exclusively for the 3'-OH and an enzyme system effecting this reaction has been characterized (Ebell *et al.* 1972). No such specificity is observed in the resins and both the 3' and 4'-OH frequently show methylation. Many roles have been suggested for flavonoids in plants (McClure 1975) and some of these functions no doubt apply in the resins. Thus the ability to absorb UV light enhances the protective nature of the coating. They may also protect the resin from microbial attack and as well, act as antioxidants to stabilise the resin.

In considering the chemistry of the diterpenoid constituents it is useful to relate them to the biosynthesis of this group (Fig. 2). It is now well established (e.g. Hanson 1972) that diterpenes along with the steroids and triterpenes arise from acetate through mevalonate leading to the C_5 pyrophosphates which provide geranyl pyrophosphate. Successive elimination-addition reactions of two isopentenyl pyrophosphate molecules then gives geranyl geranyl pyrophosphate which with some stereochemical limitations may be considered to follow divergent paths to macrocyclic ring systems such as casbene and the cembrenes, undergo cyclisation of the three terminal C_5 residues to give C_{20} analogues of the sesquiterpenes, or the all *trans* isomer may undergo cyclisation to give the bicyclic labdane ion *(1)**. Backbone rearrangement of *(1)* gives members of the *ent*-clerodane group whereas deprotonation provides copalol pyrophosphate (2). The latter may cyclise first to the tricyclic pimarene *(3)*

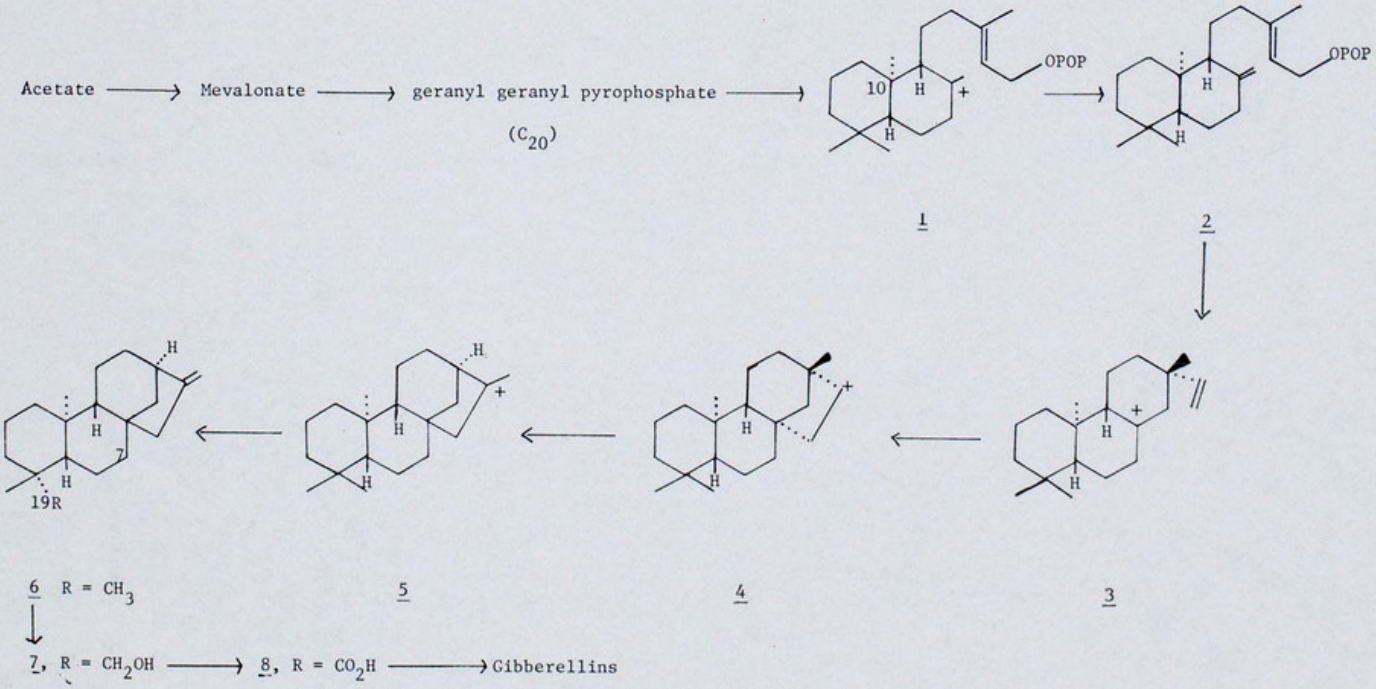


Figure 2.—Scheme for the biosynthesis of some *ent*-diterpenes.

* Numbers in parenthesis refer to structures in the figures.

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and then to the tetracyclic beyerane (*4*) and kaurane ions (*5*). The pathway is drawn to show free carbonium ions and although such species have not been proved to arise in biological systems they represent a convenient basis for rationalising the structural assembly. *Ent*-kaurene *(6)* is converted by way of the 19-hydroxy derivative (*7*) to the kaurenoic acid (*8*) which undergoes 7β-hydroxylation before generating gibberellins. In view of the widespread distribution of these latter compounds and their physiological role, the pathway to gibberellins may be regarded as part of primary metabolism in plants. The section from the bicyclic carbonium ion pyrophosphate (*1*) to kaurenoic acid (*8*) is a useful framework for discussing the diterpenes of Ricinocarpoideae since with the exception of the bertyadionol group, which are present in *Bertya cuppressoidea*, all can be regarded as deriving from intermediates on this pathway. The diterpenes of many other species are similarly derived. It is noted specifically that the stereochemistry of C-10 in this series is enantiomeric to that in the steroids, triterpenes and common pine resin acids. For the diterpenes, divergence occurs for the cyclisation of geranyl geranyl pyrophosphate which can give either the bicarbocyclic labdane (*1*) or its mirror image.

Bicarbocyclic diterpenes

The bicarbocyclic ion (*1*) is the parent of a wide range of substances which were obtained and rational pathways linking them, together with their distribution among the species are shown in Fig. 3. Hydration of *1* and pyrophosphate hydrolysis gives the diol (*10*) and this or the pyrophosphate are the logical precursors of the epimanoyl oxide (*11*) its 18-hydroxy (*12*) and carboxy derivatives *(13)* all four of which are found in *B. calycina*. The presence of a reductase generating the 13S configuration appears in both *B. lepidopetala* and *Ricinocarpus muricatus* leading from *10* to the diol (*14*) present in the latter, and then by hydroxylation of C-18 to the triol (*15*) found in both. Low specificity is indicated for the 13S reductase since both plants give 13S systems which arise from *9*. Thus reduction of *9* and hydroxylation and oxidation of C-18 or C-18 and C-15 give the carboxylic acids *(16)* and *(17)* respectively. Further hydroxylation of the hydroxy acid *(16)* occurs at C-16 to give the dihydroxyacid *(18)* found in *R. muricatus*. Polyalthic acid *(20)* in *R. stylosus* and the butenolide (*19*) in *R. muricatus* must arise by hydroxylation of C-16 at the 13-ene level coupled with further oxidation of the C-15 alcohol to the aldehyde in the former case and to the acid

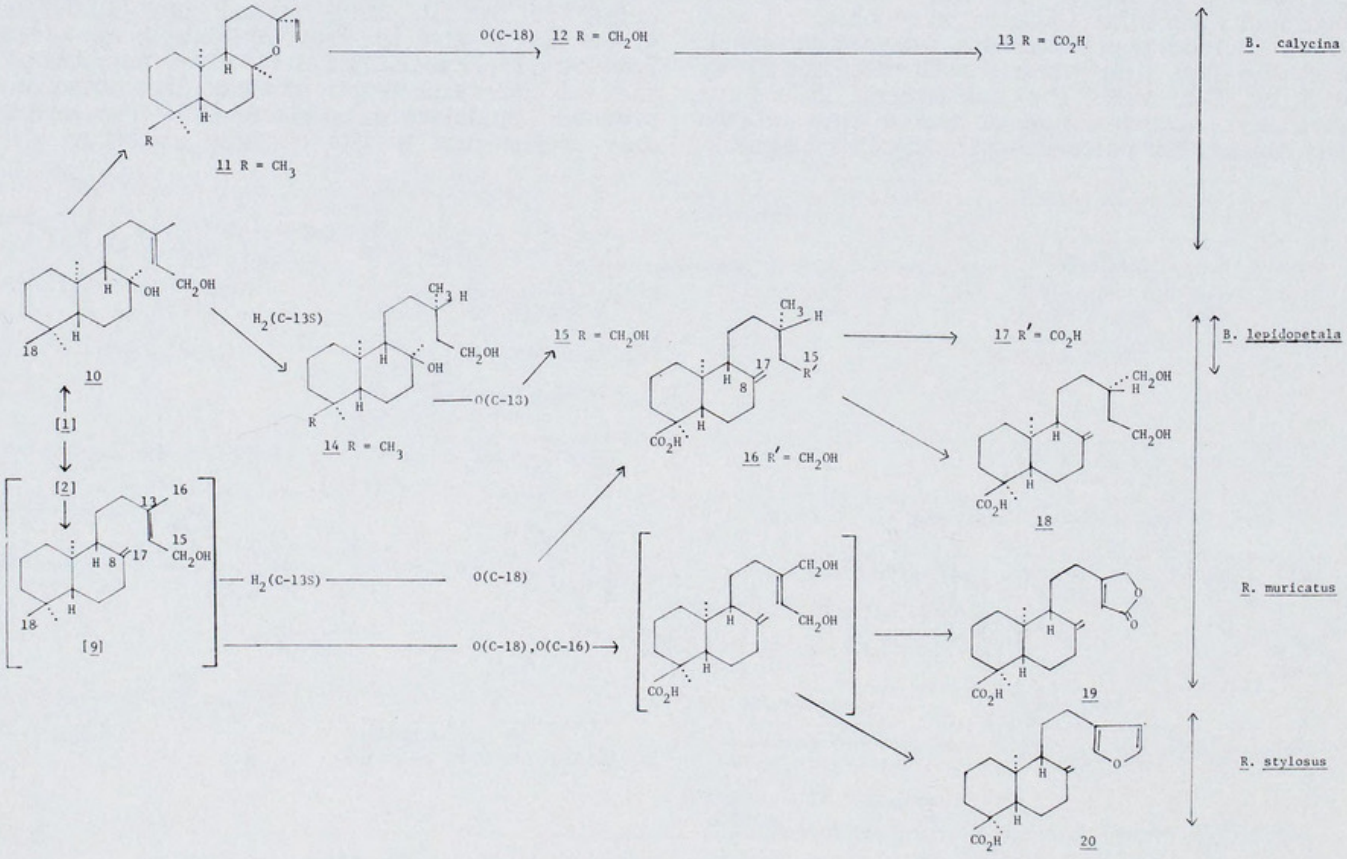


Figure 3.—Probable biosynthetic relationships of the bicarbocyclic group.

in the latter. The intermediate enediol group has been observed in diterpenes from *Sciadopitys* (Sumimoto *et al.* 1964). The hydroxylation and oxidation of C-18 in (*19*) and (*20*) might occur at any stage.

The chemical proof of the skeleton of these compounds is based ultimately on chemical interconversion with each other and with materials of known constitution, and positions of the hydroxyl and carboxyl groups follow largely from spectroscopic and pK measurements. The main ambiguity which required resolution was the configuration of the C-13 position. This was shown to correspond to that of eperuic acid which was however unsettled. We were able to clarify this situation (Henrick and Jefferies 1965a) since compounds with the 13R configuration were available to us from simultaneous research on *Dodonaea lobulata* resin (Dawson *et al.* 1966). That these were epimeric at C-13 with those from *Ricinocarpus* followed from the laboratory preparation of both from the diol (*10*). Within the various groups of polycyclic diterpenes oxygenation occurs commonly at either C-18 or C-19. The bicarbocyclic examples listed above show oxygenation at C-18 and not C-19, in sharp contrast with the tetracyclics described below, which exhibit oxygenation of C-19.

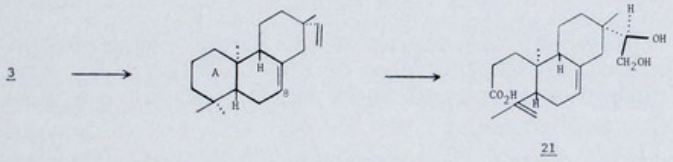


Figure 4.—Origin of the seco-pimaradiene (*21*).

Tricarbocyclic diterpenes

The families of tricarbocyclic diterpenes derive from a stereoisomer of the intermediate ion formally shown as *3*. *Beyeria brevifolia* var. *brevifolia* and an undescribed variety both generate compounds based on this skeleton. The resin of the former contains mainly one diterpene (*21*, Fig 4) obtained as ~5% of the leaves and branchlets. The efficient production of *21* is noteworthy since evidence presented in the sequel suggests that it is apparently at least four steps from the main path. Thus after formation of the 8-ene from *3*, hydroxylation of both C-3 and C-19 are evidently required before fragmentation of the A-ring can occur. Analogous A-ring splitting is seen in *Beyeria calycina* var. *minor* but has not been reported for other diterpenes although several examples are known in the triterpenes. Hydroxylation of the vinyl group could occur at any stage and is analogous to the 16,17-dihydroxylation of kaurene discussed below. The structure assigned to *21* follows from its interconversion with a derivative of beyerol.

Beyeranes

The diterpenetriol (*25*, Fig. 5) from *B. drummondii* proved to have a new carbon skeleton which was called beyerane and was recognised as the missing link (*4*, Fig. 2) in the formal pathway from the tricyclic ion (*3*) to kaurene. The structure was proved largely by classical oxidative degradation and dehydrogenation with the limited application of physical methods and the absolute stereochemistry was shown to be antipodal to the steroids by optical rotatory dispersion methods. Small quantities of the diols (*22*) and (*28*) were also isolated from a variant.

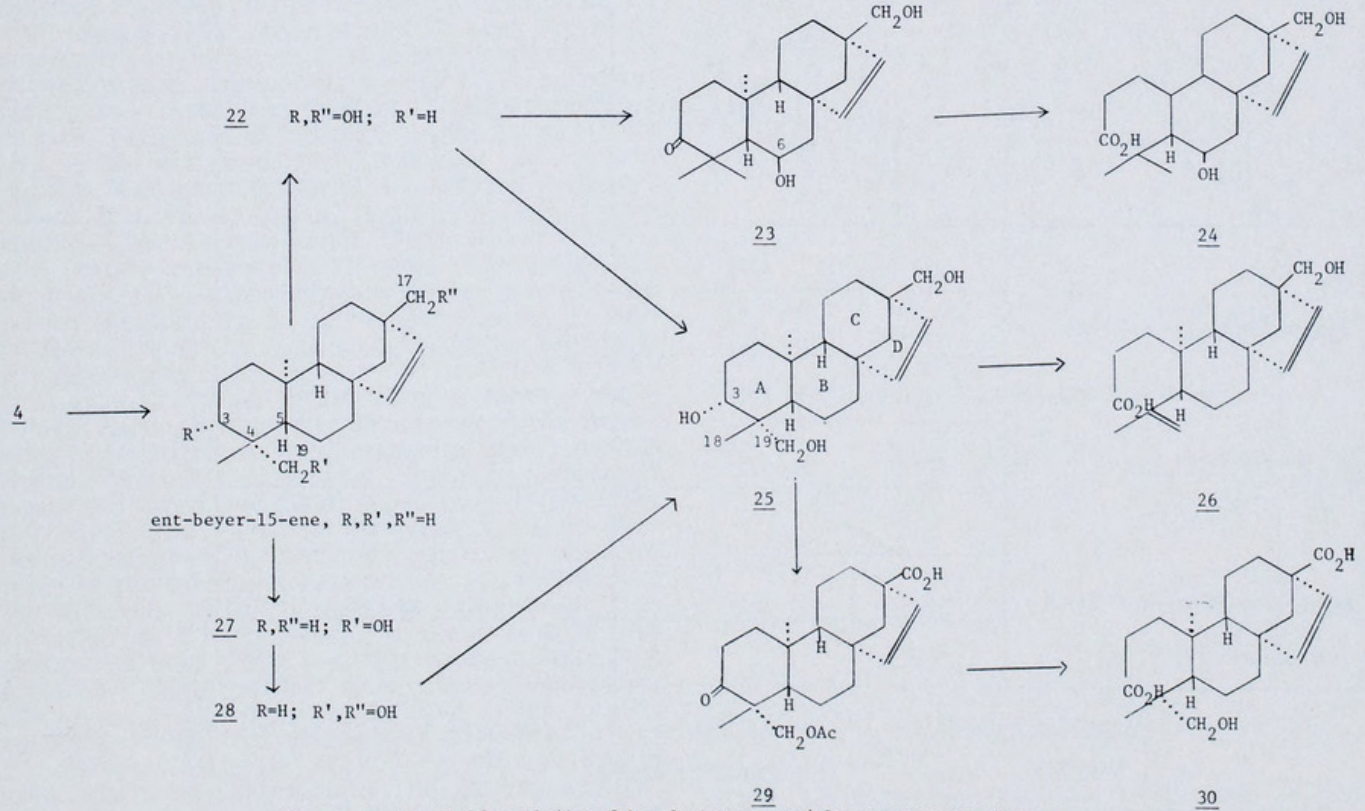


Figure 5.—Probable relationships between *ent*-beyerene metabolites.

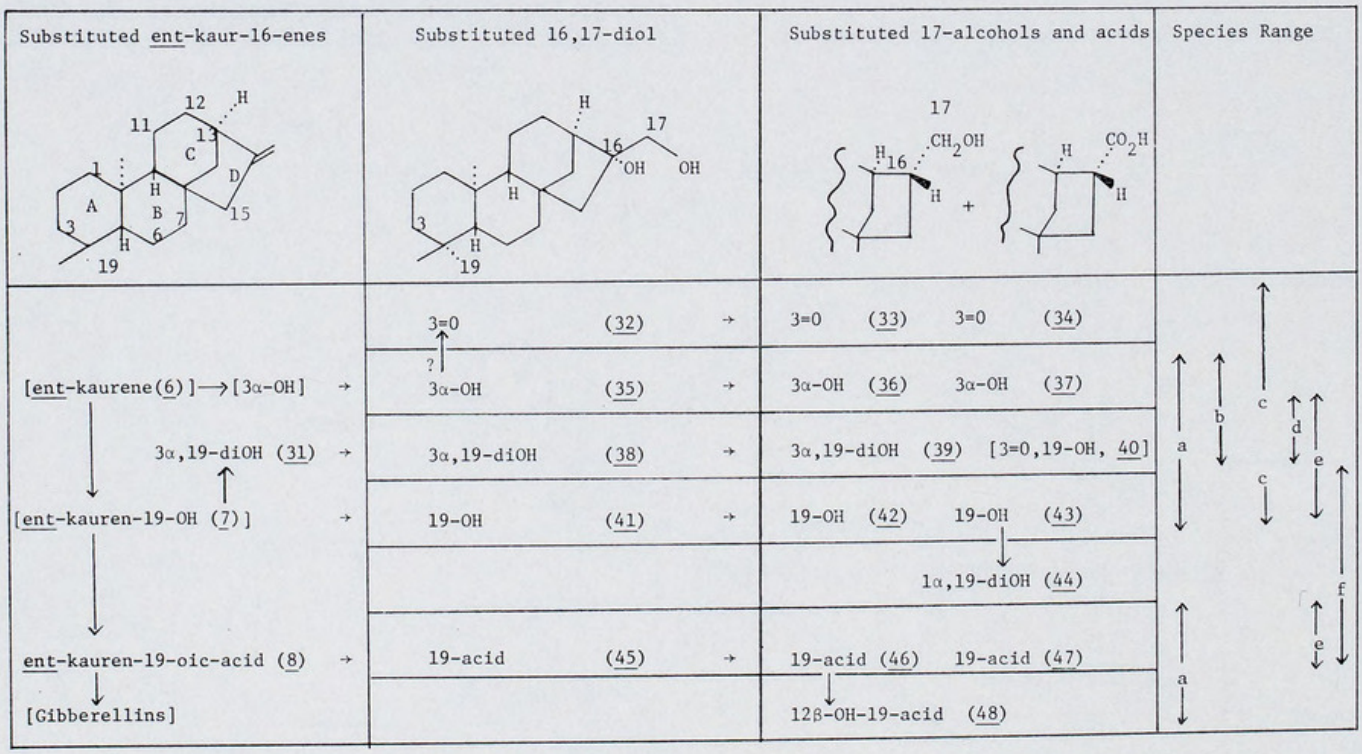
Beyeria calycina var. *minor* provided a group of beyerene metabolites of which the more abundant are the 6-acetate of the ketodiol *(23)* and the seco-acid *(26)* which has undergone A-ring fission analogous to the pimaradiene *(21)*. Several methods of effecting this ring splitting have been developed in the laboratory. Thus photolysis (Arigoni *et al.* 1960) of 3-ketones, as for example *23*, gives rise to the acids *(24)* bearing an isopropyl group as an A-ring fragment. Acids in which this residue appears as isopropenyl *(26)* can be obtained by several routes including the abnormal Beckman rearrangement (Witham 1960) of oximes of 3-ketones such as *(23)*. Both these methods were used to relate beyerol *(25)* to the seco-acid *(26)*. A biomimetic fragmentation based on the reduction of 3-keto-19-tosylates was also developed (Ghisalberti and Jefferies 1968).

Several schemes had been proposed to account for the fragmentation process in vivo although no experimental evidence in living systems was available. These schemes fell into groups either requiring C-18 and C-19 as methyl groups or those for which one of these was hydroxylated with variations within the groups arising from different oxidation states of C-3. It seemed likely that some distinction between these mechanisms might be made by feeding both *ent*-beyer-15-ene and *ent*-beyer-15-ene-19-ol *(27)* to intact plants since 19-hydroxylation is unlikely to be reversible. Seedlings of *B. calycina* var. *minor* were readily available following a bushfire and these were transplanted to a glasshouse where rapid growth occurred with regular watering. Resin production was not obviously inhibited and the normal resin components were found to be present. ^{3}H labelled samples of *ent*-beyer-15-ene, *ent*-beyer-15-ene-19-ol, *(27)*, and the 3α,19-;19,17- *(28)* and 3α,17-diols *(22)* were prepared and fed to these plants. We observed incorporation into *26* for all substrates, clearly indicating that the 4,4-dimethyl group is not essential for the fragmentation and that mechanisms based on 3,19-dioxygenated substrates are more probable (Bakker *et al.* 1972, Sefton 1978).

Beyeria calycina var. *minor* also contains small quantities of the seco-acids *(24)* and *(30)* which could be obtained independently by ultra-violet irradiation of the co-occurring 3-ketones *(23)* and *(29)* respectively. The possibility that these acids arise in the plant resin by lengthy exposure to sunlight is supported by our observation that during the short periods required for incorporation of radioactivity from the labelled substrates into *26* no significant radioactivity was detected in *24*. The retention of configuration at C4 in the sequence *29* → *30* although expected for a bio-transformation was not predicted for the photochemical reaction and is attributed to restriction to rotation of the C4-C5 bond.

Kauranes

Many of the plants we examined were found to contain metabolites of *ent*-kaurene *(6)*. The carbon skeletons of these substances were estab-



a Beyeria leschenaultii; b B. latifolia; c B. viscosa; d B. brevifolia var. brevipes
e B. calycina; f Ricinocarpus stylosus.

Figure 6.—Probable relationships between *ent*-kaurene metabolites.

lished by interconversion with each other and with the parent *ent*-16β-kaurane whose structure and stereochemistry had previously been resolved. A major problem in effecting these interconversions lay in the unsatisfactory deoxygenation methods available, particularly for hindered primary alcohols, and for this purpose we developed a technique involving the formation of benzyl thioethers from sulphonate esters using dipolar aprotic solvents (Henrick and Jefferies 1964a). Desulphurisation of these ethers to give the hydrocarbons proceeded smoothly.

A schematic relationship showing possible pathways between the oxygenated kaurenes is shown in Figure 6 together with the range of compounds associated with each species. Branching from the gibberellin pathway occurs by hydroxylation at C-3 of either *ent*-kaurene (*6*) or the kaurenol (*7*) but this is not observed for the kaurenoic acid (*8*). It is noted that C-3 and C-19 are similarly situated with respect to the D ring polar alkene group and since it is evident from microbiological hydroxylation of steroids (Jones 1973) that the distances from the binding sites control sites of hydroxylation it appears that the C-3 oxygenase and kaurene C-19 oxygenase have similar spatial demands. The C-3 and C-19 oxygenase activities operate independently since *R. stylosus* and *B. calycina* are deficient in the former and *B. viscosa* has the latter inhibited whereas the other species exhibit both about equally. The 3-and or 19-oxygenated kaurenes apparently can then undergo an unspecific hydroxylation of the double bond leading to 16, 17- diols. If hydroxylation of C-3 and C-19 is dictated by binding to the D ring double bond and if it is assumed that a binding site may in turn become a hydroxylating site (Jones 1973) then the C-3 and the C-19 hydroxyls might direct D ring hydroxylation. In the beyerenes this could explain C-17 hydroxylation and in the kaurenes 16,17-dihydroxylation can be rationalised similarly since it has been shown in metabolism of steroids by certain moulds that substitution of a saturated hydroxylation site by a double bond can lead to an epoxide (Bloom and Shull 1955) and the latter can readily generate a 1,2-diol. The formation of these 16,17-diols appears to be general and serves incidentally to remove kaurenol (*7*) and kaurenoic acid (*8*) from the gibberellin pathway. An alternative fate for the 16-ene is the formation of either the C-17 hydroxy or carboxy derivative, through steps which appear to be available to most plants examined and which permit a wide choice of substrate. Evidence presented below indicates that these substances derive from sequences analogous to the pinacol rearrangement and probably arise through the diol directly or the intermediate epoxide. In either case the aldehyde is the immediate product and this can distribute itself betwen primary alcohol and carboxylic acid. The major kaurene metabolites are thus accounted for. Two minor components are the 1α,19-dihydroxy-17-acid (*44*) which co-occurs with the 19-hydroxy-17-acid (*43*) in *R. stylosus* and the 12β,17-dihydroxy-19-acid (*48*) which co-occurs with the 17-hydroxy-19-acid (*46*) in *B. leschenaultii*. These products can be rationalised as arising from the third binding site of the hydroxylating system. Thus primary binding to the C-19 carboxyl with secondary binding to C-17 H_2OH is assumed to direct the third site to C-12β. Primary binding to C-17 carboxyl with secondary attachment C-19 H_2OH could then result in C-1α hydroxylation since the C-17 $\rightarrow$ C-12 and C-1 $\rightarrow$ C-19 distances are similar.

In summary then in its simplest terms the secondary metabolism process in Figure 5 can be explained by the operation of one hydroxylating system with three binding hydroxylating sites along with an isomerase responsible for the rearrangement of the 16,17-diols leading to C-17 alcohols and acids.

The formation of acid and primary alcohol functions at C-17 has little biological precedent and warranted further study (Croft *et al.* 1978). Five possibilities which may be considered are shown in Figure 7. Mechanism (a), an anti-Markovnikoff hydration has been postulated for a similar conversion of *ent*-kaurene in barley seeds (Murphy and Briggs 1973). Mechanisms (b_1) and (b_2) involve pinacolic rearrangements either through the epoxide or its diol and have strong chemical analogy. Mechanism (c) has its analogue in the dioldehydrase reaction and permits such exchange of H_1 on the enzyme complex (Frey and Abeles 1966). Mechanism (d) involves a dehydratase reaction leading to an enol. The mechanisms predict four different fates for the vinyl protons in *8* during its transformation to

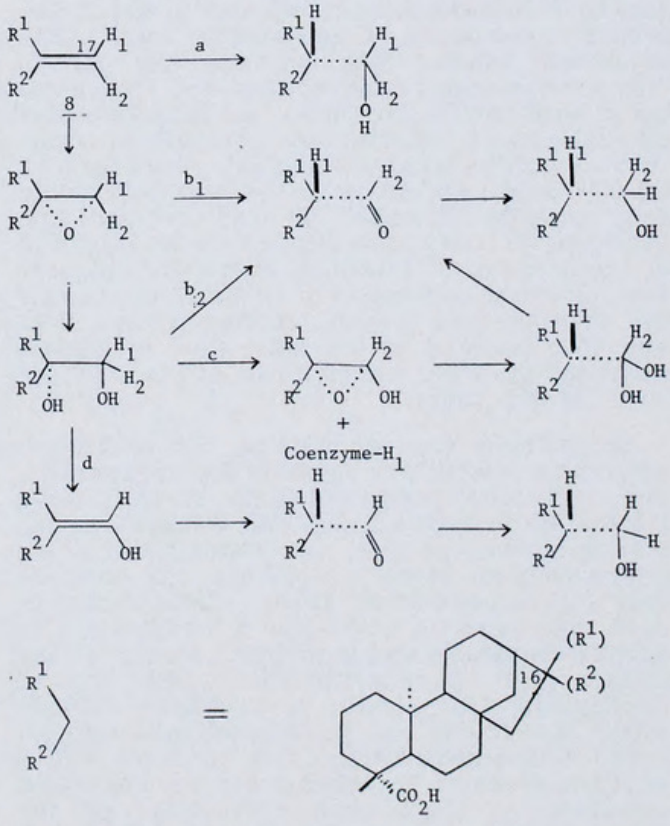


Figure 7.—Mechanisms for the biosynthesis of the hydroxy acid (*46*) from *ent*-kaurenoic acid (*8*).

46. To distinguish these mechanisms kaurenoic acid labelled with ^{14}C and ^{3}H at C-17 was prepared and fed to seedlings of *B. calycina*. The results show that no tritium was lost thus excluding mechanisms (c) and (d), and that about half of the tritium had migrated to C-16 consistent only with mechanisms of type (b). Distinction between routes (b_1) and (b_2) has not been made.

Diterpenes of Bertya cuppressoidea

It has recently become clear that a number of species of Thymeleaceae and Euphorbiaceae, particularly *Euphorbia* and *Croton* produce diterpenes which do not derive from the bicyclic ion *1*. Some of these are potent co-carcinogens, of which the esters of phorbol (*54*, Fig. 8) are best known (Hecker and Schmidt 1974).

The pathway to the carbon skeleton of phorbol has not been established but clues to a likely route are provided on the one hand by the structures of the *Bertya* diterpenes (Ghisalberti *et al.* 1973a, 1974) and by the related lathyrol group (Opferkuch and Hecker 1973) and on the other by casbene *(49)*. The latter is produced by an enzyme preparation of *Ricinus communis* (Euphorbiaceae) acting on geranyl geranyl pyrophosphate (Robinson and West 1969). Bertyadionol *(51)* is the most abundant diterpene of *Bertya cuppressoidea* and co-occurs with diterpene-D (50) and -B, the C9-ene. The structures were assigned by interrelation with each other, coupled with extensive spectroscopic studies and the absolute configuration was established by oxidation to R-methylsuccinic acid and to the cyclopropane derivative homocaronic acid. The structural relationships between the *Bertya* diterpenes strongly suggest that the five-membered ring results from aldol condensation of a diketone, which could be formed by allylic oxidation of casbene at C-8 and C-11, resulting in a C7-C11 bond. Oxidation of C-4 and C-12 would then provide diterpene D and bertyadionol. Diterpene B could arise similarly with retention of the 9-ene after inversion to the Z configuration. Further cyclisation of this skeleton to give the phorbol ring system requires linking C-12 and C-3, perhaps by an aldol type cyclisation promoted by the C-5 substituent at the aldehyde level (Worth 1971).

Bertyadionol has anomalous UV absorption suggesting interaction between the cyclopentenone and vinylcyclopropylenone chromophores. Irradiation of bertyadionol with UV light readily affords a photo product as a consequence of an unprecedented sequence leading to structure (*52*) (Ghisalberti *et al.* 1978a). The photoproduct (diterpene C) accompanies bertyadionol in the plant extracts and is probably formed in the surface resin by solar radiation. Photolysis of diterpene-D (*50*) results in an isomer (*53*) in which the 13-ene has undergone the unexceptional E-Z isomerisation. The inversion of C-2 which also occurs is probably due to ring fission generating a C-15 carbene, rotation of the C-1:C-2 bond and recyclisation as indicated in Figure 8.

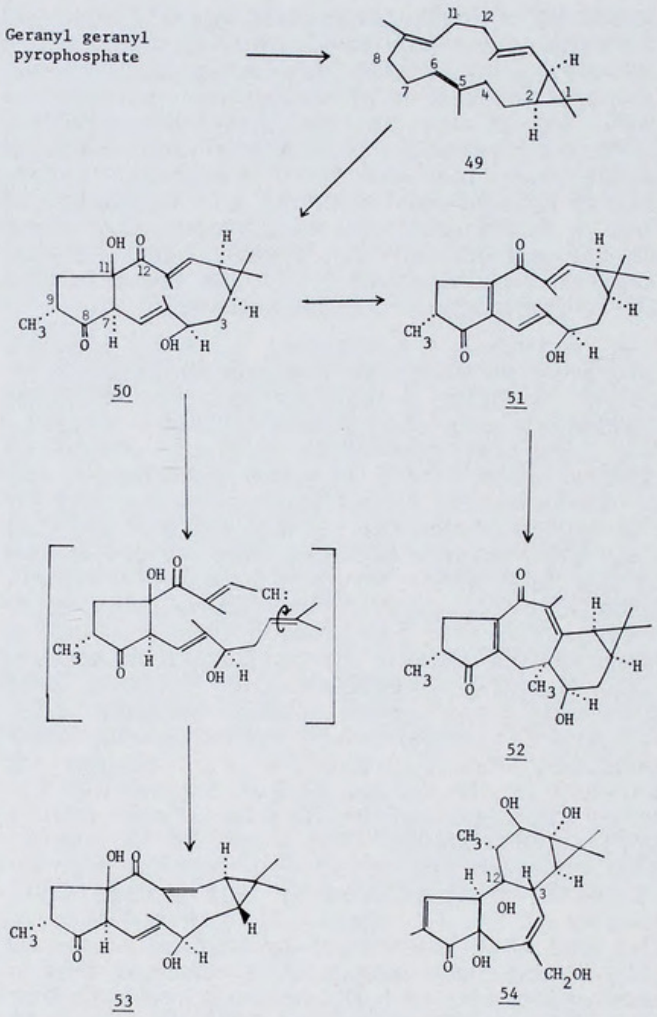


Figure 8.—Relationships between the *Bertya* diterpenes.

Mould metabolism of kauranes and beyeranes

Formal analogy between the plant and mould hydroxylating systems prompted us to carry out a survey (Beilby *et al.* 1973) of the microbial hydroxylation of a group of tetracyclics using the micro-organisms *Calonectria decora, Rhizopus nigricans* and *Aspergillus ochraceous* for which much data had been obtained on steroids by the Oxford group (Jones 1973). It was hoped on the one hand that plant hydroxylation patterns might be reproduced by appropriate mould-substrate choice and on the other we expected that the moulds in offering a wider range of hydroxylation systems might provide easy access to B and C ring hydroxylated derivatives which were not readily available from natural sources. As it turned out both expectations were realised in part (Table 2). Thus naturally occurring 1-hydroxykauranes which may be precursors of the skeleton of the grayanotoxins occur rarely but are easily available from 16,19-dioxygenated norkauranes using *R. nigricans*. Hydroxylation at C-13 is widespread in plant gibberellins but otherwise rare in natural kauranes. This step can be effected with some 16,19-dioxygenated substrates using *A. ochraceous* and although the yield is low it is comparable to synthetic procedures and much more convenient.

Table 2

*Metabolism of some kaurenes by moulds**†*

Substrate	Substrate modifications		
	Calonectria decora	*Rhizopus nigricans*	*Aspergillus ochraceous*
ent-kaur-16-en-19-oic acid (8)	7α-OH; 15α-OH; 7α, 15α-diOH	7β-OH; 16α,17-diOH	16α,17-diOH
oxygenated *ent*-16-nor-kaur-16-ones i 3α-OH	7α-OH	1α-OH; 7α-OH	6β-OH; 7αOH
ii 19-acid	7α-OH; 7β-OH; 1α-OH	7α-OH; 7β-OH; 1α-OH	13-OH; 13,16β-diOH
iii 19-OH	7α-OH; 1α-OH	7α-OH; 1α-OH	7α-OH; 16β-OH

*Beilby *et al.* 1973; Ghisalberti *et al.* 1977a. † For numbering see Figure 6.

Hydroxylation of the B ring of kaurene is prerequisite to gibberellin formation and although no B ring oxygenated metabolites of kaurene were observed in the resins 6-oxygenated beyeranes were isolated. A number of beyerane derivatives underwent 6β-hydroxylation using *C. decora* and similarly this hydroxylation technique was used to establish the skeleton of the dihydroxy acid (*29*) (Fig. 4) since the derived triol could also be obtained from the metabolism of the diol from the authentic beyerane (*27*) (Sefton 1978). All three moulds effected 7β- and 7α-hydroxylation with a wide range of kaurane derivatives and application of these and similar methods provided a simple route to 7β-hydroxykaurenoic acid (Croft *et al.* 1974), the gibberellin precursor otherwise available only by lengthy procedures. The hydroxylation by *C. decora* of kaurenoic acid gives the 15α-hydroxy derivative a process which also occurs in the plant *Phebalium rude* which contains both these substances. Another example of plant hydroxylation effected by moulds is the formation of the 16,17-diol by *A. ochraceous* from kaurenoic acid.

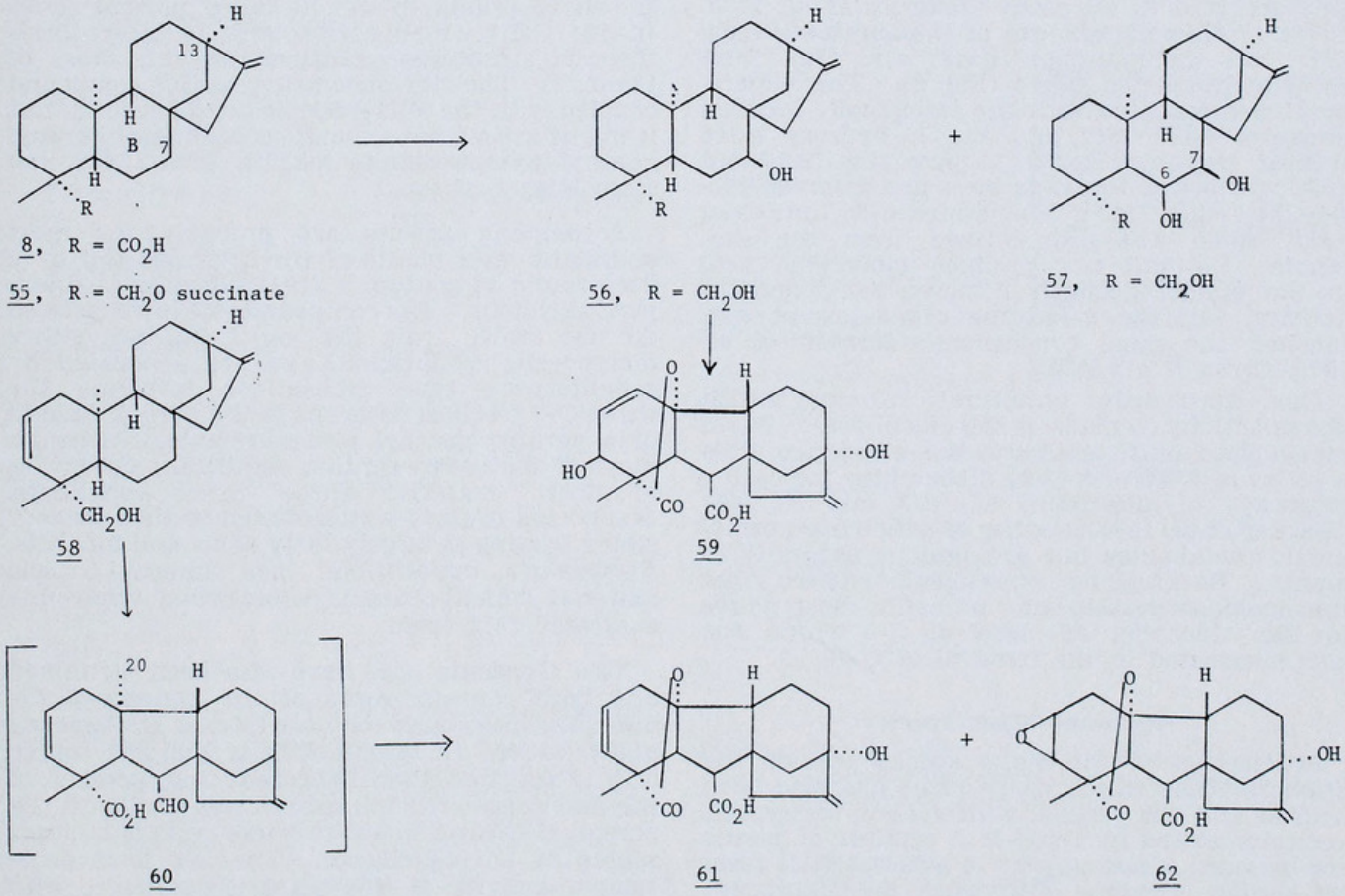


Figure 9.—Metabolism of some *ent*-kaurenes by *Gibberella fujikurci*.

Ent-kaurenes as sources of gibberellins

When kaurenoic acid (*8*) was first obtained a report had just appeared indicating that its 13-hydroxy derivative, steviol showed gibberellin activity (Ruddat *et al.* 1963) and accordingly a joint programme was established with Dr. B. O. Phinney to determine the extent of this bioactivity within our group of kaurene metabolites. Although it was found that the effect was not general for the kaurane nucleus *ent*-kaurene (*6*), *ent*-kaurenol (*7*) and *ent*-kaurenoic acid (*8*) did behave as gibberellins in a group of dwarf maize mutants (Katsumi *et. al.* 1964a, 1964b). Since *ent*-kaurene itself was known to be a precursor of gibberellic acid it seemed likely that these substances were not intrinsically active but were converted to gibberellins during assay. This proposition was supported by feeding experiments in *Gibberella fujikuroi* by several groups using material of Western Australian origin (Graebe *et al.* 1965, Verbiscar *et al.* 1967). The sequence *ent*-kaurene → kaurenol → kaurenal → kaurenoic acid → gibberellins which emerged from this work was supported by work with plant systems (Dennis and West 1967). The formation of gibberellins from *ent*-kaurenoic acid (*8*, Fig 9) clearly involved oxidation of ring B but despite some effort the appropriate metabolite had not been obtained and so a number of kaurene derivatives were fed to *G. fujikuroi* in the hope that the hydroxylation patterns obtained would identify the next step. The results implicated C-7β-hydroxylation since this occurred in all cases (Jefferies *et al.* 1970, 1974c). Thus metabolism of the succinate ester (*55*) of *ent*-kaurenol gave the 7β- and 6β,7β-hydroxylated esters (Fig. 9). This experiment was repeated with the isotopically labelled kaurenol ester (*55*) and the 7β-hydroxy ester formed was hydrolysed to give the 7β,19-diol (*56*) which was found to be a precursor of gibberellic acid (*59*). *Ent*-kaurene-6β,7β,19-triol (*57*) which was also formed from the succinate (*55*) did not produce gibberellic acid in the mould although it possesses gibberellin activity. In the meantime other groups had reached the same conclusions (Hanson *et al.* 1972, Cross *et al.* 1970).

One apparently unnatural kaurene which shows activity in maize is the dienol (*58*). When metabolised by *G. fujikuroi* this substance gives a series of C-19 and C-20 gibberellins indicating pathways to gibberellin A_5 (*61*) and A_8 (*62*) (Bakker *et al.* 1974) neither of which are normal mould metabolites but are limited naturally to plants. Backfeeding experiments showed that the epoxidation step was probably most active for the aldehyde (*60*) level of C-7 which was also implicated in the removal of C-20.

Resins of other species

A selection of the many species outside the Euphorbiaceae which carry resin has also been studied and the results, with salient references, are summarised in Table 3. A number of plants are included which appear to lack external resin but either contain diterpenes in significant quantity or belong to genera which do.

Several *Acacia* spp. are viscid and the diterpenes of *A. rossei* have been characterised as bicyclic compounds arising from rearrangement of the ion (*1*). The presence of kaurenoic acid (*8*) and its 15α-hydroxy derivative in *Phebalium rude* is a rare case of the isolation of diterpenes from members of Rutaceae. On the other hand many Western Australian species in this family produce eriostoic or eriostemoic acid (Duffield *et al.* 1962) both of which resemble diterpene acids in the possession of twenty carbon atoms, a carboxyl group and a polycyclic skeleton. Perhaps they fulfill similar roles.

Reference has already been made to labdanes in *Dodonaea lobulata* and similar compounds were found in *D. alternifolia* and *D. ptarmicifolia*. Backbone migration in the ion 1 (Scheme 1) gives the *ent*-clerodane skeleton which is the basic structure of the compounds from several other *Dodonaea* spp. Resin is commonly seen on some members of the Boraginaceae and of these *Halgania lavandulacea* has a heavy coating and was selected for study. The resin was found to be essentially a mixture of simple flavones.

Within the Discrastylidaceae the decorative *Cyanostegia angustifolia* was found to be another source of *ent*-clerodanes. *Pityrodia lepidota* has a similar component whose relative configuration was deduced by X-ray methods. The resin of *Newcastelia viscida* contains an isopimara-9(11),15-dienediol which is the only example we have encountered which has been shown to belong to the so called normal series (C-10β CH_3) which corresponds to biosynthesis through structures enantiomeric with those of Figure 1. The stereochemistry of this compound coupled with the 9(11)-double bond suggests that it might arise from anomalous folding of geranyl geranyl pyrophosphate leading to a *trans, syn* stereoisomer of ion 1.

Eremophila species are probably the most abundant resin plants of the Eremean and have been found to contain a great diversity of diterpene skeletons. The compounds we have isolated do not evolve from the ion *1* but are either macrocyclic cembranoids or are generated by sesquiterpene type cyclisations involving the three C-5 residues adjacent to the pyrophosphate of a geranyl geranyl pyrophosphate. Although most of the resins contain significant quantities of fatty material these rarely dominate. *Myoporum beckeri* is exceptional in that its very sticky coating is largely fatty acids and alcohols. *Eremophila oppositifolia* has internal viscid material which contains a branched triply unsaturated fatty acid.

Two *Goodenia* spp. have also been examined and both contain esters of the kaurene-3α,19-diol (*31*) previously obtained from *B. leschenaultii;* indeed *G. strophiolata* is the best source of 3,19-functionalised kaurenes. One member of the Asteraceae which has been well studied is the perennial *Olearia muelleri* whose resin is another source of *ent-clerodanes*. The low level antitumour activity of this plant is associated with flavone components.

Table 3

Chemical components of other resinous and allied species

Plant Species	Polyterpenes, Fatty Acids	Flavones (or Aromatics)	References
MIMOSACEAE—			
Acacia rossei F. Muell.	(8,10)-friedolabdanes	5-hydroxy-7,4′-dimethoxy-	Langley 1966
RUTACEAE—			
Phebalium rude Bartl.	kaurenoic acid (*8*) 15α-hydroxykaurenoic acid		Cannon *et al.* 1966
SAPINDACEAE—			
Dodonaea inaequifolia Turcz.	*ent*-labdanes		Payne unpublished
D. lobulata F. Muell.	*ent*-labdanes	5-hydroxy-3,6,7,4′-tetramethoxy	Dawson *et al.* 1966
D. ptarmicifolia Turcz.	*ent*-labdanes		Payne unpublished
D. microzyga F. Muell.	*ent*-labdanes		Jefferies *et al.* 1974a
D. attenuata A. Cunn.	*ent*-clerodanes		Ghisalberti *et al.* 1973b
D. attenuata A. Cunn. var. *linearis* Benth.	*ent*- clerodanes, lupanes	5,7-dihydroxy-3,6,4′-trimethoxy-	Payne and Jefferies 1973
D. boroniaefolia G. Don.	*ent*-clerodanes,	5-hydroxy-3,6,7,4′-tetramethoxy-	Jefferies *et al.* 1973
BORAGINACEAE—			
Halgania lavandulacea Endl.		5,4′-dihydroxy-7-methoxy- 5,7-dihydroxy-4′-methoxy- 5,7-dihydroxy-3,4-dimethoxy- 5-hydroxy-3,4′,7-trimethoxy-	Stacey 1970
DICRASTYLIDACEAE—			
Cyanostegia angustifolia Turcz.	*ent*-clerodanes	5,4′-dihydroxy-3,7,8-trimethoxy- 5,7,4′-trihydroxy-3,8-dimethoxy- 5,7,4′-trihydroxy-3,8,3′-trimethoxy-	Ghisalberti *et al.* 1967 Jefferies *et al.* 1973
C. microphylla S. Moore		5,4′-dihydroxy-3,6,7-trimethoxy- 5,3′,4′-trihydroxy-3,6,7-trimethoxy-	Ghisalberti *et al.* 1967
Newcastelia viscida E. Pritzel	ursolic and betulic acids isopimaradienediol (*21*)	5,4′-dihydroxy-3,6,7-trimethoxy-	Jefferies and Ratajczak 1973
Pityrodia lepidota (F. Muell.) E. Pritzel	clerodanes		Ghisalberti *et al.* 1978b
MYOPORACEAE—			
Eremophila alternifolia R.Br.		5,7-dihydroxy-3-methoxy-3,5,7-trihydroxy flavanone	Jefferies *et al.* 1962
E. clarkei F. Muell. R.Br.	cembranes		Coates *et al.* 1977 Maslen *et al.* 1977a
E. decipiens Ostf.	decipianes		Ghisalberti *et al.* 1975 Maslen *et al.* 1976
E. drummondii F. Muell.	serrulatanes		Croft 1977
E. fraseri F. Muell. (var.)	eremanes (cembranes)	5,3′5′-trihydroxy-3,6,7,4′-tetramethoxy-	Jefferies *et al.* 1962 Oh and Maslen 1968
E. georgei Diels (var.)	cembranes (eremane, preziza-enes)		Ghisalberti *et al.* 1976, 1977b, Carrol *et al.* 1976, Maslen *et al.* 1977b
E. glabra R.Br. Ostf.		(lirioresinol-B dimethyl ether)	Jefferies *et al.* 1961
E. longifolia F. Muell.		(safrole, eugenol methyl ether)	Della and Jefferies 1961
E. oppositifolia R.Br.	fatty acids		Jefferies and Knox 1961
E. ramosissima C.A. Gardn.		5,7-dihydroxy-3-methoxy-3,5,7,-trihydroxyflavanone	Jefferies *et al.* 1962
E. serrulata (A. Cunn.) Druce	serrulatanes		Croft *et al.* 1977
Myoporum beckeri F. Muell.	fatty acids	5,3′,4′-trihydroxy-7-methoxy-flavanone	Goh 1968
GOODENIACEAE—			
Goodenia strophiolata F. Muell.	kaurenediol (*31*)	5,7,3′4′-tetrahydroxy-3-methoxy-	Middleton and Jefferies 1968
G. ramellii F. Muell.	kaurenediol (*31*), labdane		Coates *et al.* 1968
ASTERACEAE—			
Olearia muelleri (Sond.) Benth.	*ent*-clerodanes	5,7,3′-trihydroxy-3,6,4′-trimethoxy- 5,7,3′4′-tetrahydroxy-3,7-dimethoxy-	Jefferies *et al.* 1974b
Helipterum craspedoides W. V. Fitzg.	beyeren-19-ol (*27*)		Dennison and Mirrington 1975

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